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双孢蘑菇遗传多样性分析*

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摘要：应用 AFLP 指纹技术对双孢蘑菇的 20 个野生菌株和 5 个栽培品种的遗传多样性进行了研究。AFLP 指纹揭示出 20 个野生异核体菌株所固有的基因型。5 个商业品种表现出比较一致的 AFLP 指纹，但也显示出它们之间的一些差别。由单孢分离获得的同核体菌株携带着部分异核体菌株的 AFLP 指纹；由同一子实体分离得到的大部分单孢菌株是异核体菌株，它们具有与其亲本一致的 AFLP 指纹。UPGMA 分析揭示出 2 个与地理分布（美洲、欧洲）相对应的组。研究结果表明：(1) 在野生菌株之间存在着明显的遗传差异；(2) 大多数单孢分离的菌株具有与母本一致的遗传物质；(3) 野生菌株间的遗传变异大于栽培品种间的变异；(4) 在双孢蘑菇的遗传多样性分析中，AFLP 技术是非常有效的工具。

关键词：AFLP 标记；遗传变异；双孢蘑菇
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Analyses on Genetic Diversity of *Agaricus bisporus*

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Abstract: Amplified fragment length polymorphism (AFLP) analysis of 20 wild and 5 cultivated strains of *Agaricus bisporus* (Lange) Imbach was done in terms of genetic diversity. The genotype destined for 20 wild heterokaryotic strains identified by AFLP fingerprints. Although 5 cultivated strains were almost identical in the AFLP fingerprints, they can be easily distinguished by AFLP markers. Homokaryons isolated from single spore carry some of the AFLP fingerprints of heterokaryon. Most strains of the single spore isolated from the same fruit body are identical with their parental heterokaryon in AFLP fingerprints. UPGMA analysis of the AFLP data reveals two distinct clusters corresponding to their different geographical populations. These results concluded (1) there are some obvious genetic differences among different wild geographical populations; (2) most strains isolated from single spore have the same genetic materials as their parents; (3) the genetic diversity of wild strains is much richer than the cultivated ones; (4) the AFLP fingerprint is an efficient tool and a new technique in genetic studies of *A. bisporus*.

Key words: AFLP markers; genetic diversity; *Agaricus bisporus*

The button mushroom *Agaricus bisporus* (Lange) Imbach (= *A. brunnescens* Peck) is an edible

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fungus. Termed secondary homothallism (Elloitt, 1972; Raper *et al*, 1972), it results in a predominance of two basidiospores basidia. This made the breeding of button mushroom suffered from an absence of out – crossing between strains and a shortage of genetic markers. As a result, commercial strains encompass extremely limited genetic resource (Kerrigen & Ross, 1989, Royse & May, 1982; Groot *et al*, 1998). Without genetic markers or reliable morphological indicator of karyogamy, such as clamp connections and mycelia appearance, the identification of strains and study on breeding become very difficult.

Recently, however, isozyme encoding loci (May & Royse, 1982; Kerrigan & Ross, 1989), restriction fragment length polymorphisms (RFLPs) (Castle *et al*, 1987, Xu *et al*, 1997, 1998) and random amplified polymorphic DNA (RAPD) (Khush *et al*, 1992) have provided simple, stable genetic markers for button mushroom. Xu *et al*, (1993) reported the mating type gene is on the largest linkage group (chromosome I) determined on the basis of RFLP analysis. Xu *et al*, (1998) had investigated the patterns of mitochondrial DNA variation in the global population of 441 isolates from nine countries in the North America and Eurasia of the commercial mushroom *A. bisporus*. Based on population genetic analysis, there are four genetically distinct natural populations in this species. In addition to isozymes, RFLPs and RAPD had demonstrated a high level of genetic diversity in wild strains, but lacking in the commercial strains, and that a source of novel germ plasm is available for introgression into the cultivated gene pool of *A. bisporus* (Castle *et al*, 1987; Kerrigan *et al*, 1990; Kerrigan & Ross, 1989; Loftus *et al*, 1988; Khush *et al*, 1992). The manipulation of genetic traits via conventional breeding schemes may, however, prove cumbersome owing to the observed lack of recombination in the organism; additional information in linkage relationships and genetic segregation in *A. bisporus* will become available with the construction of a genetic map using both isozyme and DNA markers.

Amplified Fragment Length Polymorphism (AFLP) is a new DNA fingerprinting technique that detects genomic restriction fragment and resembles in that respect the RFLP (Restriction Fragment Length Polymorphism), with the major difference that PCR amplification instead of Southern hybridization is used for detection of restriction fragment. AFLP is based on the selective amplification of a subset of genomic restriction fragments using PCR. AFLPs provide a powerful means of fingerprinting individual clones as well as establishing genetic distance and the relationships among clones that based on genetic similarities (Travis *et al*, 1996; Hill *et al*, 1996; Beismann *et al*. 1997). The DNA polymorphism identification using AFLP are typically inherited in Mendelian fashion, therefore may be used for typing, identification of molecular markers, and mapping of genetic loci (Life Technologies, 1997). Most AFLP fragments correspond to unique positions on the genome, and, hence, can be exploited as landmarks in genetic and physical maps. Each fragment is characterized by its size and its primers required for amplification. AFLP markers can be used to construct higher density genetic maps of genomes or genome segments (Vos *et al*, 1995; Karp *et al*, 1998).

To differentiate various strains of *A. bisporus* and to facilitate the breeding and genetic analysis of this species further, the author investigated the utility of DNA fingerprints generated by AFLP. In this

study, the author demonstrated that AFLP can detect the more number (restriction fragment) markers than any other DNA marker; and can be used to differentiate various strains of *A. bisporus* and to monitor transmission of genetic loci to progeny isolates.

1 Materials and Methods

1.1 Strains: The strains of *A. bisporus* used in this study are listed in Table 1. They were obtained from Horticulture Research International Cultures Collection (UK). Mycelia cultures of each strain were grown on solid CYM + CE (Xu *et al*, 1993) and in liquid CYM media.

Table 1 Strains of *A. bisporus* used in this study

Number	Strain No. and type	Origin	Number	Strain No. And type	Origin
	Wild strains		14	96.4.5	SSI of 96.4
1	ARP 174	USA	15	96.4.6	SSI of 96.4
2	ARP 116	USA	16	96.4.7*	SSI of 96.4
3	ARP 130	USA	17	96.4.8	SSI of 96.4
4	ARP 155	USA	18	96.4.9	SSI of 96.4
5	JFB Ⅱ	UK	19	96.4.10	SSI of 96.4
6	JFB Ⅲ	UK	20	96.4.11	SSI of 96.4
7	97.2	UK		Commercial strains	
8	97.3	UK	21	A15	Sylvan Co.
9	96.4	UK	22	A12	Sylvan Co.
10	96.4.1*	SSI** of 96.4	23	C3.8	Sylvan Co.
11	96.4.2	SSI of 96.4	24	2100	Amycel Co.
12	96.4.3	SSI of 96.4	25	2810	Amycel Co.
13	96.4.4	SSI of 96.4			

*: homokaryon isolates. ** SSI: single spore isolate

1.2 DNA isolation: Mycelia had been grown in liquid CYM (composition: 2% dextrose, 0.2% peptone, 0.2% yeast extract, 0.05% $MgSO_4 \cdot 7H_2O$, 0.046% KH_2PO_4 and 0.1% K_2HPO_4). Harvested mycelia were dried with filter papers (Whatman, UK) and transferred into microcentrifuge tubes. The mycelia were rapidly frozen in liquid nitrogen (−196℃) to be dried in freezer drier overnight. The dried mycelia were ground into a fine powder used inoculated needles in microcentrifuge tubes. DNA extraction was performed according to Challen *et al* (1995). DNA concentration was compared with λDNA by 0.8% agarose gel electrophoresis and diluted to 5 ngμL^{-1}. DNA samples were stored at −20℃.

1.3 AFLP analysis: AFLP analysis was performed as described in Instruction Manual of AFLP™ Analysis System II and by Vos *et al* (1995). Total genomic DNA was restricted with *Eco*RI and *Mse*I at 37℃ for 2 hours and ligate to biotinylated *Eco*RI adapters and *Mse*I adapters. The structure of *Eco*RI − adapter is:

5 – CTCGTAGACTGCGTACC

CATCTGACGCATGGTTAA – 5

The structure of the *Mes*I – adapter is：

5 – GACDATGAGTCCTGAG

TACTCAGGACTCAT – 5

Preamplification reaction was performed by using pre – amp primer mix for 20 cycles with the following cycle profile：30s DNA denaturation step at 94℃，1 min annealing step at 56℃ and 1 min extension step at 72℃. Soak temperature is 4℃. Selective AFLP amplification was then performed using three primer pairs：primer E – AG and primer M – CAG，primer E – AT and primer M – CAT，and primer E – AG and primer M – CAT. In each case，the *Eco*RI primer was labeled by phosphorylating the 5′ end with [r^{-33} p] ATP using T_4 polynucleotide Kinase. This PCR was performed by using the Perkin – Elmer 9600 thermal cycler with the following cycle profile：(i) perform one cycle at 94℃ for 30s；65℃ for 30s；and 72℃ for 60s. (ii) lower the annealing temperature each cycle 0.7℃ during 12 cycle. This gives a touch down phase of 13 cycles. (iii) perform 23 cycles at 94℃ for 30 s；56℃ for 30 s；and 72℃ for 60s. Following amplification reaction products were mixed with an equal volume of formamide dye (98% formamide，10mM EDTA bromophenol blue，xylene cyanol) for each reaction. The resulting mixtures were heated for 3min at 90℃，and then quickly cooled on ice. Each sample (2μL) was loaded on 5% denature (sequencing) polyacrylamide gel. Electrophoresis was performed at constant power，110W，for 2h. After electrophoresis，gels were dried on gel – drier，and exposed to X – ray film (Kodak Biomax® MR) through overnight. Fingerprint patterns were visualized using a Fuji BAS – 2000 phosphoimage analysis system.

The presence/absence of AFLP markers were scored by hand and coded as 1/0. Monomorphic markers were excluded from subsequent analysis which was carried out using the statistical package GENSTAT 5 (Genstat 5 committee 1993). Data from three primer combinations were combined and the pair – wise similarities calculated between samples using the Jaccard coefficient. The resultant similarity matrix was input a UPGMA cluster analysis.

2　Results

AFLPs were performed on 25 samples and showed highly informative banding pattern，which were polymorphious among strains within and between wild and commercial strains. The number of polymorphious bands among wild strains was more than the commercial strains. The number varieties of polymorphious band depende upon the primer combination used (Table 2). Altogether，329 polymorphic bands were used in the analysis.

Table 2　The relation between number of polymorphious band and primer combination used

primer combination	E – AG + M – CAG	E – AT + M – CAT	E – AG + M – CAT
number of polymorphious band	97	112	120

In addition all three primer combinations showed some putative strain – specific bands. Every pair primers produced from 25 to 35 AFLP strain – specific bands among twenty wild strains and from 10 to 20 AFLP strain – specific bands among commercial strains. The heterokaryotic SSIs AFLP patterns are quite similar to their parental AFLP pattern, In the homokaryotic SSIs (pair primers produced 25 or 37) AFLP bands, howener, are different from their parental. The two homokaryotic isolates displayed a subset of the parental AFLP bands, but homoallelic amplification polymorphism in these isolates is one homokaryotic type.

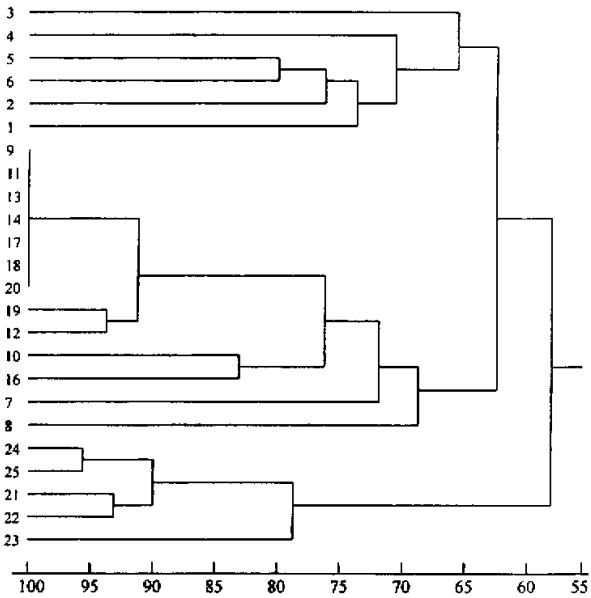

Fig. 1 UPGMA phenograms based on Jaccard coefficient among 25 strains of *Agaricus bisporus*.

3 Discussion

Using AFLP markers all strains are easily distinguished including five commercial white mushroom strains which are difficult to distinguish based upon morphological characters. The different geographical populations are easily distinguished among twenty wild strains too. The heterokaryotic SSIs AFLP patterns are quite similar to their parental AFLP pattern. The homokaryotic SSIs AFLP bands are different from their parental. The two homokaryotic isolates displayed a subset of the parental AFLP bands. The homoallelic amplification polymorphism of these isolates is one homokaryotic type. In fact, these two homokaryotic isolates are incompatible in mating reaction (data unshown).

Data analysis obtained from scoring the polymorphic band of all three primer combinations by UPGMA are shown in a dendrogram (Fig. 1) which two different groups can be distinguished. These two groups correspond to wild strains and commercial strains respectively. There are two obvious subgroups

in wild strain group and they corresponded to two geographical populations from North America and England respectively.

In order to study the sexual life cycle and its genetic diversities of *A. bisporus*, the stable, simply and effective inherited markers become very necessity. Recently, the isozymes, RFLPs and RAPDs have been proven to be invaluable in this respect (Royse & May 1982a, 1982b; Castle *et al*, 1987; Loftus *et al*, 1988; Khush *et al*, 1992). In this study, it has been demonstrated that AFLP fingerprints may be an useful tool for determining specific strains of *A. bisporus* or for assessing the relationship between strains. The AFLP fingerprints are also useful as the source for genetic markers to generate higher density linkage maps or to identify molecular markers linked to phenotype traits and /or genetic loci.

The isozyme (Kerrigan & Ross, 1989; May & Royse, 1982), RFLP (Castle *et al*, 1987; Summerbell *et al*, 1989; Hints *et al*, 1985; Xu *et al*, 1997) and RAPD (Khush *et al*, 1992) had been used to determine genetic structure of nature populations of *A. bisporus*, to confirm the crossing between strains; to detect the genetic diversities among wild and commercial strains and to analyze homokaryotic genotype isolated from single spore or from protoplast. However, in this study, it has been demonstrated that the AFLP marker technique is more sensitive and effective than others. About 100 restricted fragments of DNA of *A. bisporus* were co – amplified in each AFLP reaction and detected by denaturing gel electrophoresis. This technique is, therefore, extremely powerful for identifying DNA polymorphism, for distinguishing the strains and for generating high density genetic maps of *A. bisporus*.

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